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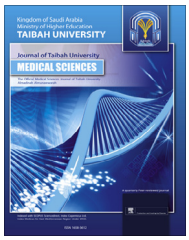

Clinical Study

Impact of estradiol monitoring on the prediction of intrauterine insemination outcome

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الملخص

أهداف البحث: يهدف البحث لتقدير القيمة التنبئية لمستويات "الإستراديول" في نفس يوم حقن هرمون الغدد التناسلية المشيمي البشري على نسبة نجاح التلقيح داخل الرحم.

طرق البحث: شملت هذه الدراسة 206 دورة تلقيح داخل الرحم تمت في الفترة ما بين يونيو 2011م وأكتوبر 2012م. سبق جميع دورات التلقيح داخل الرحم تنشيط للمبيض بهرمون الغدد التناسلية المشيمي البشري بدءا من اليوم الثالث للدورة. كما تم إجراء تلقيح واحد داخل الرحم بعد إعطاء هرمون الغدد التناسلية المشيمي البشري بـ 24-36 ساعة. واستخدم تحليل الإنحدار اللوجستي الثنائي لتحديد المتغيرات لنجاح التلقيح داخل الرحم. جرى تقييم النتيجة الرئيسة لقياس معدل الحمل السريري في كل دورة وفقا لمستوى الإستراديول .

النتائج: بحسب LR+=2 و AUC =73,0 أظهر تحليل ROC أن مستوى الإستراديول 465 بج/مل للتنبؤ بالحمل مع حساسية 60% وخصوصية 66%. حدد تحليل الإنحدار اللوجستي الثنائي وجود مستويات الإستراديول أعلى من 465 بج/مل (قيمة P أصغر من 01,0، 95% نطاق الثقة =147,0 - 687,0) ومدة التحفيز (قيمة P أصغر من 01,0 ,95% CI) =0, 201- 0, 705 كمتغيرات أعطت دلالة إحصائية لنجاح التلقيح داخل الرحم .

الاستنتاجات: مستوى الإستراديول أكبر من 465 ب ج/مل في يوم حقن هرمون الغدد التناسلية المشيمي البشري قد يشير إلى نتائج متقدمة على التحفيز المعتدل للمبيض جنبا إلى جنب مع التلقيح.

الكلمات المفتاحية: مستوى الإستراديول; التلقيح داخل الرحم; النتائج; معدل الحمل

Abstract

Objective: To evaluate the predictive value of oestradiol levels on the day of human chorionic gonadotrophin (hCG) administration on intrauterine insemination success rate.

Methods: The present study included 206 intrauterine insemination (IUI) cycles performed between June 2011 and October 2012. All IUI cycles were preceded by ovarian stimulation with gonadotrophins starting on cycle day 3. A single IUI was performed 24–36 h after hCG administration. Binary logistic regression analysis was performed to define the covariates of IUI success. The main outcome measure, clinical pregnancy rate per cycle, was assessed according to the oestradiol level.

Results: With LR + = 2 and AUC = 0.73, ROC analysis revealed out the oestradiol level as 465 pg/mL to predict the pregnancy with 60% sensitivity and 66% specificity. Binary logistic regression analysis identified the presence of oestradiol levels higher than 465 pg/mL ($p < 0.01$, 95%

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CI = 0.147–0.687) and stimulation duration ($p < 0.01$, 95% CI = 0.201–0.705) as the covariates approached statistical significance for IUI success.

Conclusions: Oestradiol level >465 pg/mL on the day of hCG administration might point out advanced outcome on mild ovarian stimulation combined with insemination.

Keywords: Intrauterine insemination; Oestradiol level; Outcome; Pregnancy rate

Introduction

Intrauterine insemination (IUI) is widely used to treat unexplained infertility and male subfertility. It is generally considered to be an intermediate step before assisted reproductive techniques such as in vitro fertilization with or without intracytoplasmic sperm injection.[1] The success rate of IUI is still a subject of controversy, with a 10–20% clinical pregnancy rate (CPR) expected per cycle.[2,3] IUI combined with controlled ovarian stimulation may increase the cumulative pregnancy rate,[4] and the low CPRs seen are often attributed to the use of mild stimulation protocols for a monofollicular response.[5] Aggressive protocols with higher doses of gonadotrophins improve pregnancy rates by increasing the number of preovulatory follicles and the rate of multiple pregnancy.[6] The aim of our study was to determine a cut-off value for the oestradiol level that predicts the insemination outcome after mild ovarian stimulation.

Materials and Methods

The population studied consisted of 206 women who underwent ovulation induction with IUI between June 2011 and October 2012. The inclusion criteria were failure to conceive for ⩾12 months of unprotected intercourse, age <38 years, basal follicle-stimulating hormone (FSH) level <12 IU/l and total motile sperm count of the partner >5 million. The exclusion criteria were ovarian cysts >15 mm on a baseline transvaginal ultrasound scan, severe endometriosis (stage III or IV according to the revised American Fertility Society staging), the total number of motile sperm <5 million, any contraindication for one of the ovarian stimulation drugs and multiple pregnancy.

All the couples had undergone a standard infertility evaluation that included medical history, physical examination, assessment of tubal patency by either hysterosalpingography or laparoscopy, basal FSH, luteinizing hormone, oestradiol, thyroid-stimulating hormone, prolactin evaluation on cycle day 3 and a semen analysis. Abnormal semen results were confirmed by a second analysis ⩾3 weeks apart. Semen samples with a concentration of >20 million/ml, progressive motility >50% and normal morphology >14% were considered normal.[7,8] The motility categories were classified as grade A (rapid linear with sluggish progressive motility), grade B (nonprogressive motility) and grade C (immotility). Semen analyses that failed to meet these criteria but with a total motile sperm count of >5 million were included. Couples were considered eligible if the woman had at least one patent fallopian tube and a documented normal endometrial cavity.

A transvaginal ultrasound scan was performed on cycle day 3. On the same day, ovarian stimulation was performed with recombinant FSH (rFSH; Gonal-F, Merck Serono, Istanbul, Turkey; or Puregon, MSD, Istanbul, Turkey) at a starting dose of 75 IU/day. If the patient's body mass index (BMI) was ⩾25 kg/m^2, the starting dose of rFSH was increased to 100 IU/day. The ovarian response and endometrial thickness were monitored by transvaginal ultrasonography first on day 7 of stimulation and then on alternate days; the rFSH dose was adjusted according to the ovarian response. When the average diameter of the leading follicle reached ⩾16 mm, 250 μg of recombinant human chorionic gonadotropin (hCG, Ovitrelle, Merck Serono, Istanbul, Turkey) was administered, and endometrial thickness and oestradiol levels were evaluated.

A single IUI was performed 24–36 h after hCG injection. The semen samples used for insemination were processed within 1 h of ejaculation by density gradient centrifugation, followed by washing with culture medium. The women rested for 15 min after IUI. Luteal phase support was given to all women with 90 mg daily of vaginal micronized 8% progesterone gel. When there were four or more follicles with a diameter ⩾16 mm or a serum oestradiol level >1500 pg/ml, hCG administration was stopped, and regular coitus was forbidden to avoid multiple pregnancy.

Statistical analysis

Statistical analysis was performed with SPSS version 16.0. The main outcome measure was CPR per cycle. Clinical pregnancy was defined as the evidence of pregnancy by ultrasound examination of the gestational sac at weeks 5–7. To predict the outcome, female age, duration of infertility, baseline FSH level, baseline luteinizing hormone level, baseline oestradiol level, total rFSH dose, stimulation duration and total motile sperm count were included in the calculations. Descriptive statistics were presented as percentages or the mean ± standard deviation. Differences between groups for categorical variables were analysed by the Chi-square test or Fisher's exact test, as appropriate, and comparisons of continuous variables between groups were analysed with the Student's t test according to the results of normality tests. Binary logistic regression analysis was used to analyse oestradiol level on the day of hCG administration, dominant follicle count, duration of stimulation, total motile sperm count before and after washing, BMI, previous pelvic surgery and insemination time, in order to identify the covariates that were significantly associated with successful IUI. Receiver operating characteristic (ROC) analysis was performed to determine the area under the curve and the likelihood ratio that showed the oestradiol level predicted pregnancy. P values <0.05 were considered statistically significant.

Results

The overall CPR of the cohort was 16.8% per cycle. The demographic and clinical characteristics of the women are presented in Table 1 and the stimulation and insemination parameters of the couples in Table 2. With a likelihood ratio of 2 and an area

Table 1: Demographic and baseline characteristics of 206 women who underwent ovulation induction with intrauterine insemination (mean ± standard deviation).

Age (years)	29.3 ± 4.9
Infertility duration (months)	53.9 ± 41
Infertility type	
Primary	145 (69.7%)
Secondary	63 (30.3%)
Body mass index (kg/m^2)	24.8 ± 4.3
Day 3 FSH (IU/ml)	6.2 ± 3.5
Day 3 luteinizing hormone (IU/ml)	5.7 ± 4.2
Day 3 oestradiol (IU/ml)	54.2 ± 37.1
Prolactin (IU/ml)	13.3 ± 8.3
Thyroid-stimulating hormone (IU/ml)	1.7 ± 3.1
Ovarian reserve (antral follicle count)	
<10	107 (51.4%)
10–20	43 (20.7%)
>20	58 (27.9%)
Menstrual cycle	
Regular	152 (73.1%)
Oligomenorrhea	51 (24.5%)
Menometrorrhagia	5 (2.4%)
Tubal patency	
Unilateral	25 (12%)
Bilateral	181 (88%)

Table 2: Type of ovarian stimulation and insemination (mean ± standard deviation).

Gonadotrophin dose (IU)	
Starting	99 ± 64
Final	105 ± 60
Total	905 ± 567
Stimulation duration (days)	9.4 ± 3.6
Motile sperm count (×10^6)	
Total	113 ± 106
After washing	90 ± 84
Oestradiol level on day 3 of hCG administration (pg/ml)	506 ± 410
Endometrial thickness on day of hCG administration (mm)	9.2 ± 1.4
Dominant follicle count	1.5 ± 0.8
Insemination time after hCG administration (h)	
24	33 (15.9%)
36	173 (83.2%)
Clinical pregnancy rate per cycle	35/206 (16.8%)

hCG, human chorionic gonadotrophin.

under the curve of 0.73, ROC analysis showed an oestradiol level of 465 pg/ml predicted pregnancy with 60% sensitivity and 66% specificity. In a comparison of the ovarian stimulation and insemination parameters of couples according to an oestradiol level <465 pg/ml vs >465 pg/ml, significant differences were found for dominant follicle count (1.1 ± 0.3 vs 2.3 ± 0.9, $p < 0.01$) and CPR (12.4% vs 23.9%, $p = 0.04$) (Table 3). Oestradiol level on the day of hCG administration, dominant follicle count, BMI, duration of stimulation and previous pelvic surgery were statistically significant ($p < 0.01$) in a regression model (Table 4). A comparison of CPR according to a dominant follicle count of 1 (10.9%) and >1 (27.3%) showed a significant difference ($p = 0.002$). No significant difference in CPR was found according to duration of stimulation.

Table 3: Main characteristics of couples according to oestradiol level on day of administration of human chorionic gonadotrophin.

Characteristic	Oestradiol (pg/ml)		*p* Value
	<465 (n = 113)	>465 (n = 93)	
Female age (years)	29 ± 4.6	29.8 ± 5.3	0.33
Infertility duration (months)	51.6 ± 34.8	55 ± 46.9	0.57
Body mass index (kg/m^2)	25.1 ± 4.4	24 ± 3.9	0.10
Day 3 FSH (mIU/ml)	6.5 ± 4.3	5.5 ± 2.1	0.07
Day 3 luteinizing hormone (mIU/ml)	5.7 ± 4.7	6.2 ± 4.0	0.48
Day 3 oestradiol (pg/ml)	53.3 ± 35.1	53.2 ± 38.4	0.99
Motile sperm count (×10^6)			
Total	109.9 ± 102.5	116.7 ± 100.5	0.67
After washing	97.5 ± 75.7	101.8 ± 78.9	0.77
Total motility (%)	66.6 ± 15	68.5 ± 16.9	0.44
Stimulation duration (days)	9.6 ± 3.2	9.6 ± 4.5	0.93
Endometrial thickness (mm)	8.4 ± 1.6	9.5 ± 1	0.05
Dominant follicle count	1.1 ± 0.3	2.3 ± 0.9	<0.01
Clinical pregnancy rate (%)	12.4	23.9	0.04

FSH, follicle-stimulating hormone.

Table 4: Factors that influence the success of insemination.

Factor	Odds ratio	95% CI	*p* Value
Oestradiol level on day of hCG administration	0.31	0.147–0.687	<0.01
Dominant follicle count	0.12	0.070–0.212	<0.01
Stimulation duration	0.37	0.201–0.705	<0.01
Body mass index	0.17	0.113–0.259	<0.01
Pelvic surgery	0.23	0.156–0.346	<0.01
Total motile sperm count	0.23	0.225–1.443	0.23
Total motile sperm count after washing	0.26	0.143–0.501	<0.01
Insemination time	0.41	0.134–1.270	0.12

hCG, human chorionic gonadotrophin.

Discussion

The results obtained show significantly higher CPRs in patients undergoing controlled ovarian stimulation with IUI with oestradiol levels >465 pg/ml and among women with two or three dominant follicles than among those with one. The overall CPR in the present study was 16.8%, consistent with the IUI success rate reported in other studies.[3,9,10] Our results confirm those of other studies of ovarian stimulation with gonadotrophins combined with IUI, with a greater probability of conception in unexplained infertility and male subfertility.[11,12]

Other studies have shown that the timing of insemination during the periovulatory period is important in order to provide sperm that are capable of fertilizing the oocyte, and scheduling IUI at different times has been studied. Improved

fecundity has been reported after double insemination performed 12 and 34 h after hCG administration and by timing insemination 60 h after hCG administration.[13] We performed a single insemination 24 h (to avoid weekends) or 36 h (on weekdays) after hCG administration and found no significant difference in the pregnancy rates between these two schedules (12.1% vs 17.9%, $p = 0.41$).

In another retrospective study, the duration of infertility was reported to affect the conception rate,[14] whereas we found no effect. Another factor associated with the success of IUI is pelvic surgery, a history of corrective pelvic surgery being a significant risk factor for poor IUI outcome.[15] In our study, pelvic surgery for appendectomy, caesarean section, endometriosis or an ectopic pregnancy had no influence on the success of IUI (OR = 0.23, $p < 0.01$), perhaps due to our small population. Gandhi et al. reported that controlled ovarian stimulation plus IUI did not improve pregnancy rates in any stage of endometriosis; however, the cumulative pregnancy rates after controlled ovarian stimulation plus IUI in stage I or II endometriosis were significantly higher than in stage III or IV endometriosis.[16]

Age is a major determinant of the success of IUI;[15] however, in our study, the mean age of the women was 29 years, and there was no difference in age between women classified according to oestradiol level on the day of hCG administration. Tomlinson et al. reported that the number of preovulatory follicles was one of the most predictive variables for the success of IUI, with a chance of conceiving of only 7.6% when only one follicle was produced and 26% with two follicles.[14] In our study, the CPR of women with two or three preovulatory follicles was significantly higher than that of women with one dominant follicle (10.9% vs 27.3%, $p < 0.01$).

It is difficult to determine the male parameters that affect the success of IUI. In cases of male subfertility, IUI has a low success rate.[17,18] Campana et al. reported that the pregnancy rate of couples with a total motile sperm count $< 1 \times 10^6$ (2.1%) was lower than that of couples with $> 1 \times 10^6$ (6.7%).[19] Another study reported a lower pregnancy rate with motility $< 20\%$.[20] A group of European investigators reported that the number of inseminated spermatozoa was the only significant predictor of insemination success.[21] A study in a Scandinavian population showed that a cut-off value of 5×10^6 for total motile sperm count was one of six predictors of IUI outcome.[18] It would be difficult to determine a universal threshold for total motile sperm count or sperm motility, and each centre should define a threshold for its population and laboratory.[9] We found a minimal influence of total motile sperm count after washing on the success of IUI. Addition of compounds such as pentoxifylline in vitro to stimulate sperm function is not widespread.[22–24]

Marviel et al. found the best results of IUI with an oestradiol concentration > 500 pg/ml on the day of hCG administration,[25] and our results are in agreement. Checa et al. reported higher pregnancy rates during IUI cycles for women who had higher oestradiol levels on the day of hCG administration.[26] Badawy compared the pregnancy rates of inseminated women stimulated with either clomiphene citrate or letrozole and reported higher rates for women with higher oestradiol levels on the day of hCG administration among those given clomiphene citrate.[27] Makkar et al. reported higher serum oestradiol levels and more follicles > 16 mm in diameter in pregnant than in non-pregnant controlled ovarian stimulation cycles combined with IUI.[28] In another study, however, the authors reported that the CPR was not affected by the number of follicles present at the time of intrauterine insemination or the serum oestradiol level on the day of hCG administration in a controlled cycle of ovarian hyperstimulation in non-andrologic, non-peritubal factor infertility, although there was a clear trend towards higher pregnancy rates with more follicles.[29]

Researchers investigated either hCG application is better before IUI or after IUI on 100 couples. The comparison of fertility rate showed no difference for different timing of hCG injections. But interestingly, the women with a history of more than 6 years of infertility had significantly higher pregnancy rate (81.82%) than the women with a history of infertility shorter than 6 years (18.18%).[30] In another study it was reported that there was no difference in simultaneous use of hCG injection compared to cycles in which IUI was performed 34–36 h after hCG injection.[31] Soria et al. reported the highest pregnancy rates in cases with shorter infertility duration, BMI $\geqslant 25$ kg/m^2, FSH < 9 IU/L, anovulation due to polycystic ovary syndrome, at least two follicles recruited through controlled ovarian hyperstimulation during IUI cycle.[32] Jeon et al. reported the unfavourable factors to predict clinical pregnancy during the first IUI cycles as follows: women older than 39 years (OR: 0.263, 95% CI: 0.076–0.906, $p = 0.034$), longer duration of infertility (OR: 0.967, 95% CI: 0.942–0.993, $p = 0.012$), endometriosis vs unexplained infertility (OR: 0.177, 95% CI: 0.040–0.775, $p = 0.022$) and endometrial thickness below 7 mm (OR: 0.114, 95% CI: 0.015–0.862, $p = 0.035$).[33]

The limitations of this study include the fact that our study population had both unexplained infertility and male subfertility. Such heterogeneity could be avoided by recruiting a study population with only unexplained infertility. Secondly, the study population was small.

Conclusion

In conclusion, IUI is a useful, cost-effective treatment for some causes of infertility. While female age and severe male factors negatively affect the success of IUI, unexplained and anovulatory infertility can be treated successfully. The serum oestradiol level on the day of hCG administration is predictive of a positive outcome, a level < 465 pg/ml indicating a poor outcome of mild ovarian stimulation combined with insemination.

Conflict of interest

All authors declare that they have no conflict of interest.

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